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A genetic screen for new mutations affecting the signaling pathway by which cells respond to TGF-beta-related factors was carried out in Drosophila. The genetic background was sensitized using a mutation in the Type I receptor. New mutations affecting the phenotypes caused by the sensitized genetic background were recovered. The new mutations were mapped to six different genees. Three of these genes were previously known to participate in the signaling pathway, confirming the ability of the screen to recover mutations in the pathway. DNA sequencing was used to establish the molecular lesion in 5 of the new mutations in the Mad gene and in the type II receptor gene. Positional cloning was used to identify the gene affected by three mutations recovered in the screen. The gene encodes a TGF-beta-related ligand called 60A. Phenotypic analysis confirmed the role of this TGF-beta-related ligand in several developmental processes in Drosophila. Recovery in the screen and the phenotypic analysis reveal that 60A may function as part of a heterodimeric ligand with the Drosophila decapentaplegic protein.

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FOREWORD

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D. Michael Huff 11/6/97

PI - Signature Date

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INTRODUCTION:

Cancer cells have multiple genetic defects that alter the cells' normal growth regulation by disrupting or activating the regulatory pathways that normally maintain an appropriate cell number in a specific tissue. Identification of the genes and proteins that participate in these regulatory pathways has provided and continues to provide insights into the molecular basis of cancer. Identification of the proteins, and the regulatory circuitry in which they participate, provides reagents for cancer diagnosis, prognostic opportunities, and targets for the discovery and development of new therapeutic agents with which to treat cancers.

Over the past 10 years, it has been established that many of the critical regulatory genes altered in human cancer cells have been highly conserved during evolution such that homologous genes exist in other organisms, including the genetically accessible organisms yeast, the nematode *C. elegans* and the fruit fly *Drosophila melanogaster.* This has provided an opportunity to use genetic strategies in these model organisms to identify new genes that function on the regulatory pathways. Dr. Hoffmann's laboratory has used this strategy to identify new components on the TGF-beta-related signal transduction cascade in Drosophila. The overall goal of these studies is to identify new gene products that are essential to the function of this pathway and to understand the molecular mechanisms by which the gene products interact. The working hypothesis, which has been confirmed now by Dr. Hoffmann's laboratory and several other laboratories, is that new genes on regulatory pathways, identified by genetic strategies in model organisms, can be used as molecular probes to identify new human genes that participate in the regulatory pathways altered in cancer cells.

RESULTS:

In the initial years of the project we identified a sensitized genetic background in Drosophila for the TGF-beta-related pathway and carried out a mutagenesis screen of 10,000 genomes for modifier mutations. Genetic characterization of the new mutations that were recovered indicated that some may be new alleles of genes previously identified on the pathway. These included five new alleles of Mad, two new alleles of punt and 1 new allele of medea. During the past year we have characterized the Mad and punt alleles by DNA sequencing. The results provided molecular confirmation of the genetic interaction and a molecular identification of the mutation as follows:

Mutant designation	Gene	Amino acid change
D14	Mad	Glutamine 168 to stop codon
D16	Mad	Tyrosine 255 to Asparagine
D24	Mad	Aspartic acid 437 to Asparagine
D13	Punt	Cysteine 67 to tyrosine
D18	Punt	Glutamic acid 378 to valine

The Mad mutation at 437 occurs in the "hot-spot" of the Mad protein that is conserved in human Smad proteins and is the region of most Smad mutations reported in human colon cancer and human pancreatic cancer. The punt mutation at 67 occurs in one of the cysteines that is conserved in human type receptor kinases.

In addition to the molecular characterization of the alleles reported above, a major effort over the past year has been the positional cloning of one of the new modifier genes.

Three alleles were recovered in a single complementation group that mapped to the distal end of the right arm of the third chromosome. Genetic crosses to stocks containing chromosome deletions were used to narrow the location of the gene to the polytene chromosome bands in subdivision 60A. A P-element transgene in that region was mobilized with transposase and a screen for P-element alleles of into the modifier gene was carried out successfully. DNA adjacent to the new P-element insertion site was cloned and cosmid clones of the region isolated. A candidate gene in the region was identified as 60A, a Drosophila TGF-beta superfamily member most similar in amino acid sequence to the human protein bone morphogenetic protein 7. To determine whether the mutations occurred in the 60A gene itself (versus in an adjacent gene), 60A DNA was amplified from the mutant flies by RT-PCR of mRNA or PCR of genomic DNA. Mutations were identified in 60A for all three sequences.

The mutant phenotype of the 60A mutations was characterized. Absence of 60A was lethal to the animal and led to absence of fat body, absence of the first constriction in the midgut and reduced gastric caeca length (structures at the anterior end of the midgut). These phenotypes are similar to those reported for mutations in the Mad gene, a protein critical for the signal transduction activated by TGF-beta-related factors.

The most interesting finding was that mutations in 60A combined with a weak mutation in the type I receptor thick veins, led to mutant phenotypes much more severe than either mutant gene alone and that the more severe mutant phenotypes were very similar to those produced by mutations in the Drosophila TGF-beta-related factor decapentaplegic (dpp). We interpret these finding to mean that dpp and 60A form a heterodimeric ligand that is important for the biological effects of both genes. The heterodimer may be largely functionally redundant to dpp homodimers. A manuscript on this work has just been submitted for publication.

Finally, two other modifier mutations recovered in the original screen are being mapped in order to pursue their positional cloning.

CONCLUSIONS:

We have successfully characterized a number of new mutations from the modifier screen to the molecular level. This includes the identification of the molecular defect present in new alleles of Mad and punt. We have identified mutant alleles of the 60A gene and discovered the importance of 60A/dpp heterodimers for Drosophila development. TGF-beta family members are known to have the capacity to form heterodimers, which may have very different functions than the homodimers, e.g., activins and inhibins, but there are few examples of these occurring *in vivo*. Our results should stimulate new interest in the existence and function of heterodimeric forms of TGF-beta-related ligands. Our progress is consistent with the goals, time table and Work Plan initially proposed.